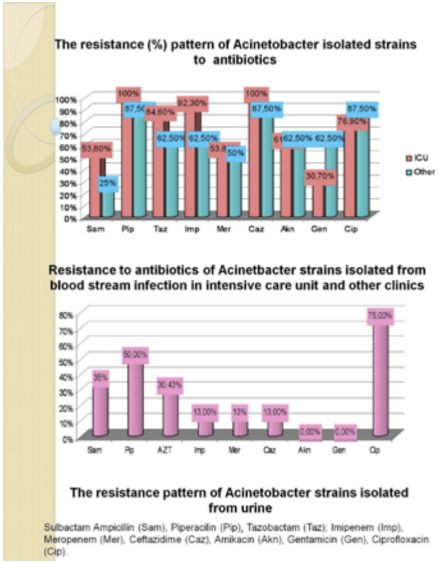

Conclusion: Aminoglycosides, Quinolones and most of $ Lactamins are no more indicated in the therapy of Acinetobacter produced infections.The presence of multiple resistant strains in ICU is attributed to invasive procedures and the use of broad-spectrum antimicrobials. It is rather difficult to distinguish morbidity and mortality attributable to Acinetobacter from that attributable to the common and severe co-morbidity in these patients (ICU). Therefore good clinical evaluation is essential to avoid unnecessary treatment. Infection control measures are crucial for limiting spread and alternative therapies with ampicillin/sulbactam are an option that needs further study.

doi:10.1016/j.ijid.2010.02.397

74.018

Real time PCR resolution of community acquired MRSA reservoirs: A strategy for the reduction of time to detection of hospital acquired MRSA

S. Connolly*, S.N. Connolly, Y. Pasari

Teesside University, Middlesbrough, United Kingdom

Background: Community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) are infiltrating hospitals and becoming the dominant colonising strains. While the optimal MRSA detection strategy remains debatable reliance on conventional microbiological methods causes delay in identifying MRSA carriers culminating in cross infection and dissemination of hospital acquired MRSA infection.

Methods: Nasal swab specimens were subjected to routine culture based and selective chromogenic screening, antibiotic susceptibility testing, as well as molecular detection using standard PCR and SYBR Green real-time PCR assays. MRSA was identified through the amplification of staphylococcal 16S rRNA, *mecA* and PVL genes.

Results: Time to detection was within 5 hours of admission using the real-time method versus 2 days for standard PCR and 4 days for microbiological methods. All hospital acquired MRSA strains carried the *mecA* gene and showed multiple resistance to a panel of antibiotics. The community source of the existing hospital strains was established through amplification of the PVL gene, identified both singularly and in multiplex PCR assays with *mecA*, demonstrating that HA-MRSA originated through the dissemination of CA-MRSA by cross infection of the carriers. PVL positive multiply resistant MRSA strains were identified from nasal specimens of healthy individuals who had not recently visited hospitals, while healthy MRSA carriers from care home facilities did not contain the PVL gene and these strains demonstrated sensitivity towards most antibiotics.

Conclusion: Thus, MRSA strains with specialised PVL-encoded virulence determinants persist in the hospital environment. This is in contrast to care home facilities where, in the absence of the selective pressure of antibiotics, low level resistance and PVL negative CAMRSA strains are selected, whereas dominance of the more virulent PVL positive MRSA is curtailed. PCR assays, particularly SYBR Green real-time PCR, of *mecA* and PVL genes are preferential procedures in contrast to conventional methods for the rapid detection of CAMRSA as a means of control of cross infection and the dissemination of HA-MRSA.

doi:10.1016/j.ijid.2010.02.398

74.019

Study of Vancomycin (VA) and Trimethoprim/sulfamethoxazole (TMP-SMX) activity on community-associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) biofilms (Bf) in vitro

A. Farinati[1,*], M.V. Campana[1], S.C. Lopez[1], R. Notario[2], J.M. Casellas[3], G. Vazquez[1]

[1] *Facultad de Medicina, Universidad del Salvador, Buenos Aires, Argentina*
[2] *Laboratorio CIBIC, Rosario, Santa FE, Argentina*
[3] *Laboratorio CIBIC, Rosario, Santa Fe, Argentina*

Background: Empirical CA-MRSA treatment could be affected by Bf development.There is an increasing appreciation that planktonic microbes account for only a very small proportion of microbial life, the bulk are found in a sessile form in Bf. Therefore, we study the influence of VA and TMP-SMX in early Bf development.

Methods: To better elucidate this, we work with 6 CA-MRSA. We employ the MIC (1.5 mg/l—0.125 mg/l) and sub-MIC (0.5 mg/l-0.06 mg/l) of VA and TMP-SMX respectively. As control we use one HA-MRSA with similar VA MIC and sub-MIC but with 20 mg/l (MIC) and10 mg/l (sub-MIC) to TMP-SMX. Aliquots of overnight cultures in tryticase soy broth were incubated with glass coupons during 3 h for cell attachement. Coupons were transferred to fresh media with and without corresponding antibiotic (AM) concentrations, incubated for 24 h and evaluation previous staining with cristal violet.

Results: Visual observations revealed that CA-MRSA isolates are less effective to form Bf than HA-MRSA. Both AMs (MIC and sub-MIC) didnŤ affect CA-MRSA but affected in different degrees HA-MRSA Bf development. Microscopically CA-MRSA with both AM produced more extracellular polymeric substances (EPS) than CA-MRSA without AM and similar to HAMRSA with or without AM. Microcolonies structures were similar in all glass coupons for all isolates. The results showed that the presence of both AM seems not to affect

early CAMRSA Bf formation and there was an increase of EPS production. Recent reports showed a relationship between VA MIC and failure among patients with MRSA bacteremia treated with VA, atributing this failure to > 1.5 mg/l MIC.

Conclusion: Our experiment might explain controversies about effectiveness of AM treatment due to Bf formation rather than presence of planktonic CA-MRSA in the patients. It is necessary to use or add other AM with activity in early stage of Bf different of VA or TMPSMX in patients with suspected or established CA-MRSA infections.

doi:10.1016/j.ijid.2010.02.399

74.020

Disc diffusion methods versus PCR for mecA gene in detection of Methicillin Resistant *Staphylococcus aureus*

Z. Mohammadtaheri[1], M. Pourpaki[2,*], F. Mohammadi[2], S. Raeissi[2], M.A. Khodadoust[2], M. Masjedi[2]

[1] *National Research Institute of Tuberculosis and Lung Disease, Tehran, Iran, Islamic Republic of*
[2] *National Research Institute of Tuberculosis and Lung Diseases, Tehran, Iran, Islamic Republic of*

Background: Accuracy and promptness in the detection of methicillin resistance are of key importance in ensuring the correct antibiotic treatment in infected patients and control of methicillin resistant *staphylococcus aureus* (MRSA) in the hospital environment. The aim of our study was to evaluate the efficacy of disc diffusion tests to characterize MRSA and compare it with oxacillin agar screening and detection of mecA gene by PCR.

Methods: Methicillin resistance of *staphylococcal* isolates derived from patients of a university hospital in Iran was assessed using the CLSI disk-diffusion method with a cefoxitin 30-!g disk in comparison with an oxacillin 1-!g disk. Oxacillin screen agar plates with 4% NaCl and 6 microg/ml of oxacillin were inoculated and interpreted as per standard guidelines. PCR-based detection of mecA gene was considered as the reference standard.

Results: Out of 200 *Staphylococcus* isolates 195 (97.5%) were methicillin resistant by oxacilline disc diffusion test, 151 (75%) were resistant by cefoxitin disc diffusion test, 153 (76.5%) were detected as MRSA by oxacillin agar test and 126 (63%) were mec A gene positive by PCR. The cefoxitin showed 96% sensitivity and 61% specificity with a positive predictive value of 80% and a negative predictive value of 92%. However, the sensitivity and the specificity for oxacillin agar test were 89% and 36%, and for oxacillin were 98% and 14%, respectively.

Conclusion: Overall, the MRSA rate is so high in our hospital with any test in this research. Comparing different phenotypic methods for MRSA screening in routine microbiology laboratory, Cefoxitin disc and Oxacillin agar screening have better sensitivity and specificity in comparison with Oxacillin disc. However, according to the different results of these tests and PCR, it seems that phenotypic expression of methicillin resistance may alter depending on the growth conditions for *S. aureus*, such as temperature or osmolarity of the medium and some other factors

besides mecA gene that may affect the accuracy of the methods used to detect methicillin resistance.

doi:10.1016/j.ijid.2010.02.400

Diagnostics (Poster Presentation)

75.001

Molecular diagnosis of *Helicobacter pylori* infection and risk factor of the presence of *cagA* and *vacA* genes

A. Hassan

Medical Research Institute, Alexandria, Egypt

Background: Several *H. pylori* genes that are related to the risk of disease have been identified. The cytotoxin-associated gene (*cagA*) is a marker for a genomic pathogenicity (cag) island of about 40 kbp whose presence is associated with a more severe clinical outcome. A cytotoxin that injures epithelial cells is encoded by vacuolating cytotoxin A gene (*vacA)*. *vacA* is present in all *H. pylori* strains and contains at least two variable parts. The aim of our study was to evaluate the efficiency of using PCR technique as a powerful tool besides being an easy and low cost method for diagnosis of *H pylori* infection through molecular detection of *cagA* and *vacAs1* genes in gastric tissue biopsies obtained from patients diagnosed as *H. pylori* positive.

Methods: Fifty cases were enrolled in our study that underwent endoscopy. The following investigations were done:

- Paraffin-embedded tissue sections were stained (H&E) to grade the severity of gastritis to detect *H. pylori* as a gold standard.
- CLO-Rapid urease test was carried out for all gastric biopsies.
- PCR analysis for detection of *cagA* and *vacAs1* genes in gastric tissue biopsies obtained from patients diagnosed as *H. pylori* positive.

Results: Biochemical results: For positive histology cases, 36/41 (87.8%) were positive, while for negative histology cases, 8/9 (88.9%) were negative by rapid urease test. PCR results: For positive histology cases, 40/41 (97.6%) were positive by the PCR test for *vacAs1 gene*. On the other hand, out of 9 that were negative by PCR test for *vacAs1 gene* 9 (100%) were negative by histology. Out of 41 positive cases by histology, only 40 (97.6%) were also positive by the PCR test for *cagA gene*. On the other hand, out of 9 that were negative by PCR test for *cagA gene* 9 (100%) were negative by histology.

Strong positive and statistically significant correlation between the expressions of these two genes *(vacAs1* and *cagA)* in patient of *H. pylori*. ($P < .000$)

Conclusion: We can conclude that:

- Rapid urease test is good screening test when multiple biopsies are used.